Coronavirus: A Possible Cause of Reduced Male Fertility

Running title: Coronavirus and male fertility

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Abstract

In lately December 2019, a novel coronavirus (SARS-CoV-2) outbreak occurred in Wuhan, PR China. It is a high contagious virus that has threatened human health worldwide. SARS-CoV-2 infection, termed COVID-19, causes rapidly developing lung lesions that can lead to multiple organ failure in a short period. Whenever a novel virus emerges, reproductive risk assessments should be performed after infection. In this review, we show that male fertility might be damaged by coronavirus associated with (i) direct cytopathic effects derived from viral replication and viral dissemination in the testis; and (ii) indirect damage to male

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fertility derived from immunopathology. In this review, we briefly describe the impaired fertility of humans and animals infected with coronaviruses to deduce the impact of the new coronavirus on male fertility. Together with information related to other coronaviruses, we extrapolate this knowledge to the new coronavirus SARS-CoV-2, which may have a significant impact on our understanding of the pathophysiology of this new virus.

Keywords: Coronavirus, Fertility, Male, SARS-CoV-2, Testis

1. Introduction

Coronaviruses are the largest family of positive-stranded RNA viruses, which includes 30 members at present. They are widely distributed in nature, including infections of humans and other mammals. In recent years, new coronaviruses have caused problems worldwide in cycles, such as severe acute respiratory syndrome coronavirus (SARS-CoV) occurring in 2002, and Middle East respiratory syndrome coronavirus (MERS-CoV) being first identified in 2012. In 2019, a new highly contagious virus broke out in Wuhan, Hubei province, China, termed SARS-CoV-2, representing the seventh member of enveloped RNA coronaviruses[1]. The 2019 novel coronavirus disease (COVID-19) caused by SARS-CoV-2 has common clinical manifestations such as fever, dry cough, and in severe cases, multiple organ damage [2-4].

Regarding the critical molecule for SARS-CoV-2 transmission, the receptor angiotensin I converting Enzyme 2 (ACE2) for virus cell entry and transmembrane serine protease 2 TMPRSS2 for priming the S protein[5], are co-expressed in the testis and male genital tract[6], which suggests a high possibility that the virus targets the testis and male genital tract during

infection. It was reported that over 25 viruses could enter human semen and negatively affect sperm or male fertility[7], such as HSV [8] and HIV [9]. Whether SARS-CoV-2 may have the same the effect on males is an important question that was not answered unambiguously in a preliminary investigation[10].

To date, studies [11-13] have confirmed the absence of SARS-CoV-2 RNA in the semen of patients with COVID-19. Conversely, The results of Li et al.'s study were inconsistent with those of previous studies and detected of SARS-CoV-2 in 6 of 38 semen samples[14]. Similarly, Yang et al. reported that one case (1/12) with a high viral load was positive for viral RNA after post-mortem examinations of testicular tissue[15], which supported the idea that high viral loads in patients with severe disease symptoms might reach the threshold to cross the blood-testis barrier[16]. On the other hand, a study showed that compared with patients with mild disease, patients with severe COVID-19 have significantly lower testosterone levels[17], suggesting that the co-expression of ACE2 and TMPRSS2 on Leydig cells might make them susceptible to SARS-CoV-2 infection and thus compromise testosterone secretion[18]. However, considering the high false-negative results for SARS-CoV-2 using RT-PCR[19], as well as the limitation of the small sample size and selection bias mostly obtained from recovering mild cases[10], we still need to be cautious when evaluating this data. Nevertheless, it is well known that coronaviruses can contribute to high morbidity and mortality in both humans and animals [20,21]. A study has demonstrated orchitis in patients with SARS, with detrimental effects in the testis, suggesting that coronavirus can infect the male reproductive tract and impair male reproduction [22]. SARS-CoV-2 and SARS-CoV share some common clinical manifestations, which supports the hypothesis that the new coronavirus might directly infect the testes and

male reproductive system. Therefore, we should be vigilant about the impact on male reproduction in patients with COVID-19. In addition, the blood-testis barrier might allow the testes to act as a special reservoir to protect viruses against antiviral agents[23], which is a key reason for considering the testes as a particularly important organ for study in the context of the SARS-CoV-2 pandemic, and it is especially important because the coronavirus family has been identified the culprit causing orchitis in both humans (SARS-CoV)[22] and animals (feline coronavirus and avian coronavirus) [24,25]. Using evidence from previous studies of coronavirus-infected animals and humans, the implications of this review may help us to understand the impact of SARS-CoV-2 on male reproductive capacity.

2. Direct virus-induced cytopathic effects

Various viruses can replicate in the male reproductive tract, such as HEV [26] and ZIKV [27,28], which eventually lead to testicular atrophy and male infertility. Viral infection of the male genital tract can provide insights into possible male fertility impairment after SARS-CoV-2 infection. SARS-CoV-2 enters cells by binding ACE2 and via priming by TMPRSS2. ACE2 is a membrane-associated secretase that is expressed primarily on endothelial cells and is the host cell receptor for SARS and SARS-CoV-2 [29-31]. Notably, ACE2 is highly tissue-specific, with significant levels being detected only in the heart, kidneys, testes, and gastrointestinal tract [32-34]. In the testes, ACE2 is expressed only in spermatogenic cells and testis somatic cells, suggesting a high potential for testicular damage and spermatogenesis disruption when the virus combines with this metalloprotease[35]. TMPRSS2, as an essential protease for viral infection, is highly expressed in spermatogonia and spermatids[18]. The co-expression of ACE2 and TMPRSS2 in spermatogonia and Leydig cells

implied that the testis might be a high-risk organ that is vulnerable to SARS-CoV-2 infection, which might result in testicular degeneration and male infertility. SARS coronaviruses, whose expressed proteins share 76% amino acid sequence identity with those of SARS-CoV-2, were detected in testis somatic cells [36]. This observation supports the hypothesis that the SARS-CoV-2 might concentrate on testis cells to dysregulate their function.

2.1 Direct virus-induced damage of the testis

Viral replication in cells contributes directly to microscopy-detected lesions, which eventually result in spermatogonia necrosis [26], such as in a ram model of infection by Bluetongue virus (BTV) (an arbovirus of ruminants), which showed testicular parenchyma damage and the destruction of the Sertoli cells caused by viral replication-induced cytopathic effects [37]. Coronaviruses might use a similar mechanism in humans to impair male fertility. ACE2 is the crucial determinant of coronavirus infection, tissue tropism, and subsequent viral replication[38,39]. The expression pattern of ACE2 in adult human testis at the level of single-cell transcriptomes was shown to be predominantly enriched in Leydig and Sertoli cells[6]. Besides, alternative receptor Basigin (BSG) and protease Cathepsin L (CTSL) were also detected in Leydig cells[40], which can mediate SARS-CoV-2 into cells. Data from autopsies of 12 patients with COVID-19 showed a dramatic reduction in Leydig cells in the interstitium[15], supporting the speculation that SARS-CoV-2 could display tropism for Leydig cells, ultimately leading to ultrastructural lesions and decreased numbers of Leydig cells. Leydig cells occur in clusters between blood vessels and seminiferous tubules, producing the vast majority of androgens in men [41]. The replication of SARS-CoV-2 in testosterone-producing Leydig cells might disrupt testosterone production. Indeed, a recent

study confirmed that patients with COVID-19 suffered hypogonadotropic hypogonadism as the disease the progressed, implying that the secretory function of Leydig cells might be impaired by the novel coronavirus[17]. Testosterone is essential to preserve male fertility and to support Sertoli cell maturation and the development of Leydig cells [42]. Extensive evidence from clinical and laboratory studies implied that testosterone deficiency is accompanied by atrophy of the testicular parenchyma and degradation in the seminiferous tubules [43,44], in summary, testosterone is necessary for men to maintain the blood-testis barrier, spermatogenesis, and fertility. Alterations in male sex hormone levels induced by SARS-CoV-2 might negatively affect male reproduction. Therefore, special attention should be paid to andrology examinations and hormone assessments on men recovering from COVID-19, as well as exploring the possible long-term outcomes of SARS-CoV-2 infection.

Sertoli cells are the only somatic cells in the tubules that directly contact with spermatogenic cells, and control the differentiation of spermatogenic cells via paracrine signals [45]. Inhibin B is secreted by Sertoli cells, and compared with follicle-stimulating hormone (FSH) or luteinizing hormone (LH), it is an ideal marker for spermatogenesis and a better indicator of sterility [46,47]. SARS-CoV-2 has a high affinity for human ACE2, which suggests that the virus might concentrate on Sertoli cells. Indeed, inhibin B levels decreased after hepatitis E virus infection in mice, which was attributed to damage of the Sertoli cells in the testes [26]. Accumulating evidence suggests that the coronavirus family has an affinity for these testes cells, for example, Avian infectious bronchitis virus (IBV), a subtype of coronavirus, can cause acute respiratory infections in birds [48], and was detected in Sertoli cells of the testes of infected roosters using immunofluorescence [49]. Roosters vaccinated with live

attenuated IBV showed significantly reduced serum androgen concentrations compared with non-vaccinated roosters and could cause infertility in roosters [50]. Considering that IBV causes a similar severe acute respiratory syndrome to SARS-CoV-2, we hypothesized that the same mechanism might be used by SARS-CoV-2 to spread in Sertoli cells. In addition, roosters vaccinated with live attenuated IBV might provide an animal model of how SARS-CoV-2 replicates in cells and causes pathogenic effects in the testis.

Additionally, coronavirus might directly disrupt the microenvironment of the testis that supports spermatogenesis. In CoV-infected roosters, histological analysis revealed the disruption of seminiferous tubules and loss of the basement membrane, leading to the destruction of the spermatogenesis microenvironment, which contributed to the reduction of the live sperm concentration [51]. Thus, testicular degeneration is possibly the result of several overlapping factors once a coronavirus infects the male genital tract, and this might also be the case for SARS-CoV-2.

2.2 Virus-induced damage of spermatogenesis directly

Viruses can be detected in semen directly. SARS-CoV-2 RNA has been isolated from rectal swabs and respiratory tract swabs[52]. Currently, the question of whether the virus can infect semen needs an answer. According to a recent study of scRNA-seq data in adult human testes, ACE2 and TMPRSS2 are highly co-expressed in spermatogonia, which are enriched in the gene ontology (GO) categories relating to viral reproduction and transmission[6]. Therefore, it is reasonable to hypothesize that there is a high risk of SARS-CoV-2 presence in seminal fluid[53]. However, a few case reports have investigated this issue, and the presence of SARS-COV-2 in semen remains ambiguous[11-14]. Notably, gene ontology (GO) enrichment

analysis illustrated that cell junction and immunity-related GO terms were enriched in ACE2-positive Leydig/Sertoli cells; therefore, cell-cell junctions might allow the transfer of SARS-CoV-2[6], which might represent one explanation of the highly contagious nature of this novel coronavirus and could have implications for sexual and reproductive behavior[54]. Taken together, there is a critical need to verify virus infection semen and whether sexual transmission of SARS-CoV-2 can indeed occur.

With regard to research on coronavirus-infection animals, IBV has been isolated from testicles and semen in roosters [51,55], and when insemination using IBV-spiked semen was performed, IBV RNA could be detected in all the hens, and the weight of eggs laid by the hens inseminated with IBV-spiked semen was significantly reduced [55]. Knowledge of other coronaviruses present in semen might encourages researchers to look at semen and sexual transmission, to determine whether SARS-CoV-2 can be sexually transmitted like IBV; however, the results will need to be cautiously interpreted. Nevertheless, we should remain vigilant to this possibility, which has important implications in reproductive medicine, especially viral transmission facilitated by ART, such as intracytoplasmic sperm injection (ICSI)[56], sperm cryopreservation, and the prevention of transmission. During this epidemic, sperm cryobank must introduce precautionary measures: first, we recommend that semen from SARS-VOV-2-positive men is cryopreserved in a highly secure, separate container, such as a vapor cryostorage tank. Secondly, all donors must undergo mandatory SARS-COV-2 testing. Thirdly, abstinence or condom use might be considered as preventive measure for patients with COVID-19.

Virus binding to ACE2-expressing spermatogonia would disrupt spermatogenesis[6].

Rooster vaccination with coronavirus caused a significant reduction in daily sperm production [25,57]. A recent report also confirmed that semen quality parameters were impaired in patients with moderate infection of COVID-19[58]. Hence, the risk of SARS-CoV-2 virus infection to semen parameters may not be negligible. Notably, the long-term impact on semen parameters of SARS-CoV-2 infection, as well as semen examination, is required during follow-up patients recovering from COVID-19, especially men who plan to have children.

2.3 Direct virus-induced damage of the epididymis

In animal models of coronavirus infection, one of the major clinical symptoms is epididymal stone formation [51,59,60]. IBV replication in roosters' testes might result in severe cellular micropathological damage, which in the long-term can lead to the presence of epididymal stones. The presence of stones is associated with reduced fertility and adverse effects on sperm function[61], eventually resulting in the collapse of the seminiferous tubules and cessation of spermatogenesis. The epididymis is a crucial region for sperm maturation, which is pivotal for sperm to obtain the motile ability and fertile capacity. Dysfunction in this area can compromise sperm maturation and further impair sperm quality, such as decreased sperm motility, increased DNA damage, changed membrane lipids, and the acrosome reaction[62]. If the behavior of coronavirus infection in humans is similar to that in animal models, we should pay attention to the epididymis to protect it from SARS-CoV-2-induced destruction.

In view of these result, we suggest prompting a comprehensive genitourinary examination for patients with COVID-19, including alterations in semen parameters, such as the acrosome reaction, DNA damage, and sperm motility.

3. Indirect immune-mediated damage to male fertility

The testicle is an immunologically privileged organ, the blood-testis barrier (BTB) protects the testes against pathogen invasion [63]. In healthy fertile men, various immune cells and cytokines produced by non-immune cells are indispensable to ensure male fertility[64], in which they maintain the testicular microenvironment balance and male reproductive health within the intricate and active environment of the seminiferous epithelium. Testicular tissue development benefits from immune cells and their cytokines, and the immune response is critical to control and eliminate viral infection[65]. Cytokines are important for the immune response to viral infections by regulating the expansion and location of leukocytes. However, infection and inflammation might disrupt the immune balance in the body, either through immune insufficiency or overactivation, possibly leading to devastating effects in humans[66]. Immune pathology associated with an uncontrolled immune response might give rise to testicular parenchyma destruction when the BTB is damaged by virus infection[67], and any associated functional impairment could lead to male infertility.

3.1 Cytokine-mediated infertility

SARS-CoV-2 has proven effects on multiple organs throughout the body [68], accompanied by immunopathological reactions and high cytokine storms. In the plasma of patients with COVID-19 in intensive care units, higher plasma levels of cytokines were detected, implying that a cytokine storm might aggravate the infection in patients with COVID-19 [2,3]. Actually, this coincided with the research that patients with COVID-19 presented a typical profile of hyper inflammation, such as TNF-α, IL-6, and IL-1β[69]. Cytokines are beneficial to testicular function and sperm production, as well as testicular

immunity privilege [70-72]. However, a high concentration of inflammatory cytokines could contribute to the progression of sexual dysfunction[73]. Thus, a change in cytokine production can lead to fertility problems[66,74]. Cytokine-mediated suppression of the hypothalamic-pituitary-testicular axis could lead to a decrease in serum testosterone, such as IL1 leading to inactivation of the P450/c17 lyase that converts progestins into androgens in immunopathogenesis, which will result in decreased testosterone and sperm production [60,75-77]. This corroborated the results showing a dramatic decline serum testosterone in 17 patients with severe COVID-19, which might even predict poor progression of COVID-19 infection[78]. With a history of COVID-19 disease, SARS-CoV-2 infection can attribute to male hypogonadism[79], thus it is recommended to measure testosterone levels when a patient is detected as positive for SARS-CoV-2 RNA and conduct appropriate testosterone treatment if necessary. Studies detected dramatic increases in IL6 levels in patients with COVID-19 [80,81]. Immunopathologically, high IL6 expression correlates with a systemic inflammatory milieu that disrupts the integrity of the blood-testis barrier[82]. As a result of blood-testis dissemination, the virus might damage testicular tissue directly. Furthermore, COVID-19-induced changes to the cytokine microenvironment might even lead to testicular cancer[64], which could have long-term adverse effects on the recovery of patients, and represents a second long-term matter of concern. Hence, it should be noted that the cytokine storm introduced by SARS-CoV-2 could be associated with immunopathogenesis, which might contribute to testicular dysfunction and reduced male fertility. Nevertheless, this hypothesis requires follow-up confirmation, and the exploration of possible short- and long-term consequences on their andrological health.

3.2 Inflammation-mediated infertility

The blood-testicular barrier might not be a perfect barrier to viruses under systemic or local inflammation[7]. To eliminate the virus infection, an inflammatory cytokines storm can recruit leukocytes, resulting in inflammation characterized by leukocyte infiltration in the interstitial tissue of the testes, which, as a feature of human testicular orchitis, might lead to male infertility. Actually, there is a high risk of that men with SARS-CoV-2 might suffer from an orchitis-like syndrome[35]. Pan et al. confirmed that six patients (19%) with COVID-19 suffered from orchitis [11]. Recently, a study of 12 deceased patients with COVID-19 also revealed viral orchitis characteristics, with T lymphocyte intrusion into the testicular parenchyma, accompanied by significant seminiferous tubular injury[15]. Interestingly, the histopathological features of the testes in patients with SARS also overlap with those in patients with COVID-19: All testes being full of leukocyte infiltration and wide-ranging germ cell deterioration, with thickened basement membranes [83], which supports the hypothesis that the coronavirus-induced adaptive immune response might play a vital role in the course of testicular damage and eventually affect fertility. Theoretically, attributed to the hypercoagulable state of vasculitis in patients with COVID-19, the testicular damage could be result of testicular segmental vascularization[84]. One study has shown evidence of direct SARS-COV-2 infection of endothelial cells and diffuse endothelial inflammation[85], Endothelial dysfunction may be subsequent to organ ischemia[86], which might provide a rationale for one study that described ischemia-related priapism in a patient with COVID-19 [87], suggesting that vasculitis-orchitis might have a crucial role in the development of the testicular injury caused by SARS-CoV-2 infection. Moreover, the intrusion of CD68+

macrophages into the interstitial tissue of the testes could contribute to a decline in steroidogenesis and testosterone[66], and the change in the hormonal profile might contribute to susceptibility to SARS-CoV-2 infection, leading to a more profound pathophysiological role in COVID-19 patients [88].

During the SARS-CoV-2 outbreak, SARS-CoV-2 infected-cats also presented a profile of testicular atrophy [89], and were reported to have acquired the infection from humans [90]. Furthermore, studies on chickens infected with coronavirus IBV also showed that immune cells infiltrated into the interstitium of the testis, which was responsible for the reduced fertility [25,91].

In summary, the coronavirus-induced adaptive immune response might lead to testicular damage and endocrine abnormality, eventually disrupting spermatogenesis in patients recovering from COVID-19. However, this hypothesis remains to be confirmed and studies should be undertaken to establish an animal model to determine the underlying pathophysiological mechanisms and to mitigate the risk of testicular injury during COVID-19 disease. Precautions against SARS-CoV-2-induced male infertility should be taken.

3.3 Antibody-mediated infertility by SARS-CoV-2

In SARS-infected testes, Immunohistochemistry analysis showed a large amount of IgG precipitation in the seminiferous epithelium of the testis, as well as in degraded germ cells and Sertoli cells, suggesting that the extensive IgG triggered by a secondary autoimmune response might aggravate the testicular damage [22]. In addition, deposits of IgG are associated with autoimmune orchitis (EAO)[92], which might activate immune cells in the host to produce antibodies against the virus, as well as introducing antibodies into semen[93]. In patients with

COVID-19, the positive rate of IgG reached 100%[94], especially antiphospholipid antibodies[95], which are antisperm antibodies that could interfere with fertilization[96], suggesting that male patients with COVID-19 should be cautioned against the adverse effects of a high IgG titer on their reproduction ability.

In healthy testis tissue, immune cells and cytokines are beneficial for the development of spermatogonia. However, the immune imbalance associated with infection and inflammation can contribute to male sterility. Overall, in addition to the pathogenic effects of coronavirus, the host-induced immune response against the virus also plays an important role in the overall disease process.

3.4 High fever and steroid-mediated infertility

It is generally believed that high fever can be detrimental to the normal function of the testes. Fever is one of the notable features of COVID-19 [3], and thus might play an important role in testicular dysfunction. Germ cells can develop at a normal pace at temperatures less than 37.8 °C; however, higher temperatures might cause irreversible damage to germ cells. Research confirms that high temperatures can lead to the meiotic arrest of germ cells [97]. In general, increased body temperature has a negative influence on spermatogenesis and may ultimately lead to male infertility.

In addition, patients with COVID-19 were almost all affected by SARS-CoV-2-related stress and were advised to use steroids (methylprednisolone, 1–2 mg/kg per day)[2] to treat SARS-CoV-2; however, the stress[98] and corticosteroid therapy might have adverse effects on sexual function, such as reduced libido and erectile dysfunction [99,100]. Leydig cells were also proven to be dysfunctional in glucocorticoid-treated rats [101]. Therefore, these observations

suggest that the assessment of fertility in patients with COVID-19 is imperative.

4. Conclusion

This review obtained clues from basic research on other viruses to understand how the novel SARS-CoV-2 virus might generate pathogenic effects in male fertility. We highlighted that male fertility might be highly vulnerable to SARS-CoV-2 infection. Infection with this novel virus not only seriously threatens an individual's overall health, but also might lead to male infertility. Perspectives gained from multi-organ research during the recent epidemic raises the possibility that damage to the male reproductive tract might be an underappreciated result of SARS-CoV-2 infection. Therefore, more attention should be paid to the effects on male fertility of SARS-CoV-2 infection, and should this causal link between SARS-CoV-2 infection and male infertility be confirmed, male patients should consider cryopreserving their sperm to preserve fertility.

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Authors' roles

Wenbing Zhu and Chuang Huang conceived and designed the study. Chuang Huang and Xiren Ji drafted the manuscript. Wenjun Zhou, Zhenghui Huang, Xiangjie Peng, Liqing Fan, and Ge Lin revised the drafts. All authors approved the final version of the manuscript.

Conflict of interest

None declared.

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